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Extended Summaries Pesticides in Food and Drink

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Criteria for Evaluating Laboratories for their Involvement in the Italian Monitoring Network of the Ministry of Agriculture on Pesticide Residues in Food

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Introduction

In 1992 The Italian Ministry of Agriculture, Food and Forest Resources (MiRAAF) started a programme to monitor residues of pesticides in agricultural products, with the aim of obtaining data on the levels of contamination resulting from normal agriculture practice.[1,2]

The analyses were not aimed at compliance with official regulations, that being the responsibility of the Ministry of Health and its local organs (Local Health Boards: USL), but at identification of those crops and active ingredients with the greatest residues risk, at determining the entity of the residues from plant protection chemicals used in agricultural practice and, on the basis of the data obtained, to take any necessary actions, either organisational or regulatory.

Unlike monitoring schemes in most other European and non-European countries, the samples of fruit or vegetables to be analysed are taken not from retail or wholesale markets but directly from the farms at the time the various crops are harvested. Each sample is accompanied by a record sheet listing all the technical information on the farm, the type of crop protection programmes practised and the treatments carried out during the whole period of vegetative growth.

During the three years in which the programme has been in operation, 28 crops have been monitored and more than 35,000 samples of fruit and vegetables analysed.

It was not possible to use government analytical facilities for the purpose because of certain organisational and operational problems, so private-sector laboratories were used. These had to meet certain standards essential for this type of monitoring. The task of identifying and selecting laboratories with the facilities and skills required was entrusted to the Istituto Sperimentale per la Patologia Vegetale (ISPaVe) of Rome.

Another task entrusted to ISPaVe was that of promoting the creation and growth of qualified laboratories in areas where they were lacking, thus providing adequate methodological and operational support. The aim was to achieve complete coverage of the national territory with laboratories, suitable and validated by a single organisation, that could act as reliable points of reference both for the programme of residues monitoring, and for investigations requested by other operators (farmers or farmers' associations, and those involved in trading or promoting farm produce).

Methodological notes

Identification of the laboratories to be included in the network took place in two stages:

(1) Preliminary evaluation of the laboratory activities in terms of good laboratory practice (GLP) and on the basis of specific requirements for the monitoring programme.

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(2) Results of analyses on samples similar to those envisaged for the monitoring programme proper; these involved certain pesticides as analyte and homogensied fruit as the matrix.

The laboratories were first examined on the basis of documentation supplied by themselves and of technical visits. This examination covered official recognition (e.g. by the Ministry of Health etc.) of the laboratory's standard of good practice. If there was no such certification available (which was the situation for most of the laboratories at the time), a check was made on general compliance with the rules of GLP with which the laboratory would have had to comply to obtain such certification. Additionally, the laboratory had to be near enough to the sampling point for fast delivery of the product to be analysed, so as to avoid deterioration of both the product and the active principles sought. The laboratory also needed to be big enough, in terms of both equipment and personnel, to enable a great number of samples to be analysed, and the analytical organisation to be sufficiently flexible to cope with fluctuations in the amount of work resulting from samples being taken at the different times when the various crops were ready for harvest.

A final and fundamental requirement was the ability of the laboratory to provide results promptly, so that the farmer would be able to dispatch produce for sale on Italian or foreign markets with a knowledge of its residues status.

At the start of the programme, this time was set at seven days, but this was later reduced in many cases to two to three days.

The entry test consisted of determination of a certain number of active principles from one or more chemical groups to be included in the monitoring programme (Table 1). These were mixed in fruit puree. However, laboratories in the Centre, South and Sicilia areas were given samples in which the active principles were in acetone solution. Each laboratory was left free to use multi-residue methods and procedures habitually used for residues analysis, the aim being to exploit existing experience with the laboratory's own equipment, there often being substantial qualitative and quantitative differences in this equipment.

TABLE 1
Pesticides Surveyed with the Monitoring Programme

Benzimidazoles	*Carbamates*	*Acaricides*
Benomyl	Carbaryl	Amitraz
Carbendazim	Ethiofencarb	Bromopropylate
Thiophanate-methyl	Pirimicarb	Clofentezine
	Propoxur	Dicofol
Dichloroanilines	Methomyl	Endosulfan
Chlozolinate		Hexythiazox
Iprodione	*Dithiocarbamates*	Propargite
Procymidone	Mancozeb	Tetradifon
Vinclozolin	Maneb	
	Metiram	*Organophosphorus*
Thiophthalimides	Propineb	*Pesticides*
Captan	Thiram	Acephate
Chlorothalanil	Zineb	Azinphos
Folpet	Ziram	Azinphos-methyl
		Chlorpyrifos
Anilides	*Pyrethroids*	Chlorpyrifos-methyl
Dichlofluanid	Alpha-cypermethrin	Diazinon
	Cyfluthrin	Dimethoate
Pyrimidines	Cypermethrin	Fenitrothion
Fenarimol	Deltamethrin	Fenthion
Nuarimol	Fenpropathrin	Heptenophos
	Fenvalerate	Malathion
Conazoles	Flucythrinate	Methamidophos
Bitertanol		Methidathion
Cyproconazole	*Benzoylurea*	Omethoate
Dichlobutrazole	*Insecticides*	Parathion
Exaconazole	Diflubenzuron	Parathion-methyl
Myclobutanil	Teflubenzuron	Phosalone
Penconazole	Triflumuron	Phosphamidon
Propiconazole		Pyridaphenthion
Triadimefon		Quinalphos
Triadimenol		Trichlorfon

Particular attention was given to the preparation of the test sample. On the basis of preliminary trials, it was decided to add to a finely ground and weighed (fruit) matrix standard solutions of the selected active ingredients in acetone. These were pesticides for which it was known that reproducible results were attainable. The mixture was then agitated in a homogeniser for a considerable time, giving a standard puree. Before preparing the samples to be delivered to the laboratories, five bulk samples were taken for a test of uniformity of distribution of the active principles in the matrix. This test was performed in our laboratories.

For each test, samples (250 g) of the puree were frozen ($-20°C$) and coded and then delivered to the laboratories together with a blank sample containing only the matrix. Three of these samples, chosen at random, were on each occasion held at the ISPaVe chemical laboratory for a further test of concentration of active principles after storage at $-20°C$ for different lengths of time followed by thawing for analysis. The samples were delivered to the laboratories by ISPaVe or by various monitoring centres in their respective areas.

The laboratories were notified 15 days in advance of the arrival of the samples, which were delivered together with a record indicating the chemical groups (organophosphorus, carbamates etc.) within which the search for the active principles added should be conducted. They were instructed to send, with the results of the analyses: a description of the methods used, pointing out the individual values obtained; the chromatograms for the samples and standards for all the active principles in the grid of the chemical group in question; and the pattern of calculations made for quantification. The average time allowed for the laboratories to send in their results was seven working days. Using the same criteria, other 'confirmation during operations' tests were performed at intervals on the laboratories included in the Residues Monitoring Network once their suitability had been recognised.

Assessment criteria

In both the entry test and the confirmation tests, the responses from the various laboratories were coded to make them anonymous to the examiner and then assessed on a criterion based on calculation of the acceptable percentage difference from the true value for the residue; this percentage (P) is related to the true value (v) by the function

$$\log P = 2 \log 5 - \log 2 \times \log v$$

defined for the interval $0{\cdot}01 \text{ mg kg}^{-1} \leq v \leq \text{mg kg}^{-1}$ and

$$P = 100 \text{ for } v < 0{\cdot}01 \text{ mg kg}^{-1} \text{ and}$$

$$P = 25 \text{ for } v > 1 \text{ mg kg}^{-1}$$

according to criteria set out for analysis of residues in EEC Directive 91/414 adopted in Italy by the Decree Law of 17 March 1995.[3]

The concept of acceptable reproducibility of the results obtained from repeated analyses of a sample by a laboratory (intra-laboratory) was thus transposed to the results obtained by different laboratories on the same sample (inter-laboratory). The yardstick for assessing the tests, and therefore the suitability of the laboratories for inclusion (initial or continued) in the network, was obviously based on the type and size of the errors made in the analytical determinations and on logistical considerations in relation to the monitoring network.

Examination of the results of the tests—in which the laboratories in the different areas were asked to take part from time to time—resulted in creation of a merit ranking scale based on:

1. Absence of errors of false negatives;
2. Types and number of quantitative errors;
3. Number of errors of false positives.

On the basis of this ranking scale, the laboratories were admitted to the monitoring network according to a yardstick that also took account of other needs such as: the number of analyses forecast for the area served by the laboratory; the number of analyses the laboratory was able to perform; and the desire to stimulate development of analytical services in certain areas of the country where they are in short supply. The result was that in some decisions a laboratory that produced even a single false negative (which carried the highest penalty in the assessment score) was not included in the network. In other cases, laboratories that had made an error of this type for a single active principle were admitted to the monitoring network if the other errors were mostly within the interval of acceptability.

Territorial distribution of the laboratories

In the North Area (the Regions of Lombardia, Liguria, Piemonte, Valle d'Aosta, Trentino Alto Adige, Friuli Venezia Giulia, Marche, Emilia Romagna, Veneto) coordinated by the Centro Operativo Ortofrutticolo of Ferrara, the number of laboratories has risen from the initial nine to the present 22.

With the setting up of other Monitoring Centres to cover the rest of the country, additional laboratories were identified in these areas on the basis of the same criteria. These are not always uniformly distributed geographically, because of differences in the demand for tests on agricultural produce. Since the laboratories are generally in the private sector, operating according to the laws of the market, they tend to be concentrated in parts of the country where there is more demand for their services and thus in areas of modern intensive agriculture where producers are interested in exports

(and the certificates they require) and in areas where the health authorities are more active.

In the Emilia Romagna Region, for example, there is a concentration of laboratories of such size and potential that they could, if necessary, make good the shortages in other parts of the country.

There are also a good number of other organisations working in the residues area in the Regions of Lazio, Campania and Sicilia.

Results of the tests

The following data are shown for each test performed: the theoretical value (v); acceptable error for each active ingredient (E), calculated as $E = p \times V$; error as difference from the theoretical value for each active ingredient determination supplied by the laboratory (ε).

The data in Table 2, from a number of laboratories, illustrate the variability of results of the first four tests carried out in the North Area. The false negative errors were all from laboratories which did not become included in the network. The overall improvement in accuracy from the first to the fourth tests can be attributed to an improvement in methods and equipment used and to greater experience.

Results for analyses performed in the Centre, South and Sicilia areas are summarised in Table 3 with results for 'entry' tests omitted because they involved standard solutions and not a matrix. Comparison of results in Column A, those for all participating laboratories, with

TABLE 2
Percentage Distribution of Analytical Results obtained by the Four Initial Tests carried out in Northern Italy.

Active ingredients	*Error type*[a]	*Test* 1	2	3	4	*Average*
Organosphosphorus pesticides	I	64·0	72·7	93·3	86·6	79·2
	S	36·0	17·3	6·7	13·4	18·8
	FN	0·0	8·0	0·0	0·0	2·0
Carbamates	I	—	50·0	87·5	92·3	76·6
	S	—	26·0	10·0	7·7	14·6
	FN	—	23·0	2·5	0·0	8·3
	I	—	76·6	—	—	76·6
Dichloranilides	S	—	20·0	—	—	20·0
	FN	—	3·4	—	—	3·4
Conazoles	I	—	84·0	—	—	84·0
	S	—	16·0	—	—	16·0
	FN	—	0·0	—	—	0·0

[a] I = error (ε) $\leq$ acceptable error (E); S = error (ε) $>$ acceptable error (E); FN = false negatives.

TABLE 3
Percentage Distribution of Analytical Results obtained by the Tests carried out in Different Areas[a]

Active ingredients	*Error type*[b]	*Central Italy* A	B	*Southern Italy* A	B	*Sicily* A	B	*Total* A	B
Organophosphorus pesticides	I	50·0	53·3	56·7	66·7	38·8	77·8	48·4	63·6
	S	44·6	43·3	30·0	33·3	25·0	22·2	35·2	34·8
	FN	5·4	3·4	13·3	0·0	36·2	0·0	16·4	1·5
Benzimidazoles	I	50·0	65·0	60·0	100·0	50·0	100·0	54·0	73·1
	S	50·0	35·0	40·0	0·0	33·3	0·0	43·2	26·9
	FN	0·0	0·0	0·0	0·0	16·7	0·0	2·8	0·0
Carbamates	I	50·0	55·0	25·0	30·8	33·3	50·0	34·6	45·5
	S	36·0	40·0	35·0	46·2	22·2	33·3	30·.8	40·9
	FN	14·0	5·0	40·0	23·0	44·5	16·7	34·6	13·6
Dichloroanilides	I	50·0	65·0	—	—	—	—	50·0	65·0
	S	50·0	35·0	—	—	—	—	50·0	35·0
	FN	0·0	0·0	—	—	—	—	0·0	0·0

[a] A = data supplied by all laboratories taking part in the test; B = data supplied by the laboratories included in the network.
[b] I = error (ε) $\leq$ acceptable error (E); S = error(ε) $>$ acceptable error (E); FN = false negatives.

results in Table 2 for the North Area indicates a clear difference in quality. Results from the Centre and South areas are inferior and this may be attributable to less experience and poorer equipment.

However, results in Column B of Table 3, which are from those laboratories assessed as suitable for the network, show much less disparity between the North and other areas. These results have helped to stimulate increased professionalism and greater investment by laboratories in areas where the need for health and hygiene tests on farm produce has hitherto been less marked.

The detailed examination has also revealed those molecules which create most problems in analysis. The results for acephate, azinphos methyl and methomyl are outside the limits of acceptability in 44·7, 35·6 and 26·6% of the analyses respectively. There was also a high percentage of false negatives for methomyl (23·3%) which may be due in part to the low level of this compound in the test sample which was of the same order as the legal limit of 0·02 mg kg^{-1} tolerated by Italian regulations. This active principle can be determined by HPLC using the usual detection system (UV) only above a certain concentration. The increased sensitivity in analytical procedures required to detect the low concentration of the chemical present in the test samples can be achieved only by insertion of a post-column derivatiser on line. Only a few laboratories had this costly piece of equipment at the start of the monitoring programme.

It is considered advisable to conduct a study, through inter-calibration tests, on the causes of inaccurate analysis of this and other active principles and to attempt to determine whether such errors are random (attributable to the operating quality of the analysis units) or systematic (due to the inadequacy of the methods of analysis adopted).

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